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Effect of Almond Intake on Fecal Fat Excretion in Healthy Adults

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Effect of Almond Intake on Fecal Fat Excretion in Healthy Adults

by

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A Thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science in Nutritional Sciences

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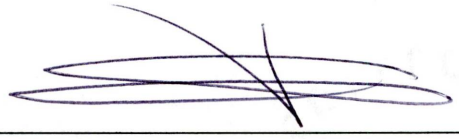
Each person whose signature appears below certifies that this thesis in his/her opinion is adequate, in scope and quality, as a thesis for the degree Master of Science in Nutritional Science.



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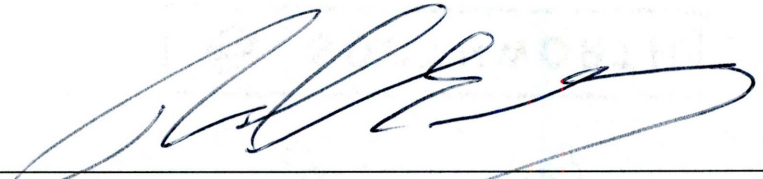

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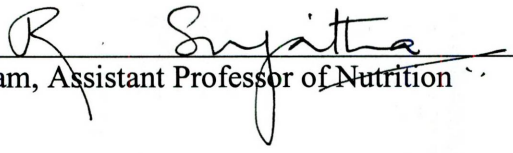

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ABSTRACT OF THE THESIS

Effect of Almond Intake on Fecal Fat Excretion in Healthy Adults

by

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Master of Science, Graduate Program in Nutrition Science
Loma Linda University, June 2005
Dr. Joan Sabaté, Chairperson

Background: Caloric consumption in excess leads to weight gain. Consuming nuts, in particular almonds, on a regular basis would likely increase overall caloric intake due to their high fat content, assuming complete digestion and absorption. However, multiple studies report no significant change in body weight when subjects consume nut-rich diets.

Objective: The objective of this study is to measure the effects of almond consumption on stool composition, particularly individual fatty acid content.

Design: Subjects participated in a randomized, crossover, controlled feeding study. Following a 2-week run-in period on a typical American diet (34% energy from fat), subjects were randomized to the Step I diet, low almond diet and high almond diet (0%, 10% or 20% isoenergetic replacement of Step I diet with almonds respectively), for four weeks each. Stool samples were analyzed for total fatty acid and individual fatty acid content.

Results: There was a significant increase in total fat and individual fatty acids excreted on diets with greater amounts of energy from almonds (P-trend <0.005). Total stool fat (P<0.001), palmitic, oleic and linoleic fatty (P<0.05) acids excreted differed significantly on the high almond diet when compared to control and low almond diets, with no considerable change from control to low almond intake. Stearic acid excretion increased on high almond diet (P<0.01), while no significance was detected comparing control with low almond intake or low almond with high almond diet.

Conclusions: The amount of total fat and individual fatty acids excreted is greater on diets providing 20% of energy as almonds compared to a Step I diet. The fatty acid profile in stool reflects the fatty acid composition of almonds. Excretion of fat, particularly from almonds, may explain why weight balance is achieved with daily consumption of ~68 g of almonds.

CHAPTER ONE

INTRODUCTION

Statement of the Problem

Nuts have been a dietary staple around the world for thousands of years. They are packed with nutrients, including protein, fiber, vitamin E, magnesium, zinc, copper, phosphorus and potassium, with a large amount of the calories coming from fat. A majority of the fat, however, is in the form of unsaturated fat. Most nuts are rich sources of monounsaturated fat, the primary fatty acid being oleic acid.

Prompted by the low prevalence of cardiovascular disease in countries consuming a monounsaturated-rich Mediterranean diet, extensive research has been conducted investigating the relationship between monounsaturated fats and the risk of developing cardiovascular disease. As a result, there is now conclusive evidence showing that the intake of monounsaturated fats reduces the risk of cardiovascular disease (Fraser et al 1992).

Recently, researchers have been looking at the effects of foods rather than individual nutrients on the risk of cardiovascular disease. Nuts have become a popular test food due to their high proportion of monounsaturated fat. Recent literature demonstrates that nuts have the same cardio protective effects as monounsaturated fats (Sabaté et al 2003 suppl), providing evidence that a diet rich in nuts can be beneficial to one's health.

Despite the current pro-nut message, many weight-conscious individuals remain wary of adding nuts to their diet due to their high-fat content. With the 'low-fat' health message having been engrained in the minds of many people, any food labeled as high fat is often restricted. Unfortunately, categorizing nuts as such prevents individuals from gaining their demonstrated cardio protective effects.

What if the total amount of fat in nuts was not fully absorbed by the body, either due to the inherent make-up of the nut or some factor interfering with its complete digestion? Investigators have stated that during controlled, human nut feeding studies subject's weight did not change significantly (Sabaté et al 2003). Perhaps there is incomplete absorption of dietary fat on nut-rich diets, with more fat being excreted in the feces.

In this study we seek to identify biochemical indicators of nuts, in particular almonds, in human feces in hopes of shedding light on the digestibility of these nuts. To date, there have been no published studies on this subject. We hope our results will alleviate the concerns associated with a high fat, nut-rich diet and explain the lack of weight gain reported in published studies using nuts as test food.

Purpose of the Study

Objectives

1. To assess the absolute and relative amounts of fat in stool samples of subjects while on varying levels of almond intake.

    a. Hypothesis: Stool samples collected during the almond diets will have increased absolute and relative amounts of fat.

2. To examine the effects of almonds on the fatty acid profile in stool.

    a. Hypothesis: The fatty acid profile of stool samples from almond diets will reflect the fatty acid profile of almonds, particularly the primary monounsaturated fatty acid found in almonds, oleic acid.

CHAPTER TWO

REVIEW OF THE LITERATURE

Dietary Fat

Dietary fat is an important source of energy for the body. Since fat has an average of 9 kcal/g, it is more energy dense than carbohydrate and protein. More than 95% of dietary fat is in the form of long-chain triglycerides, with most of the remaining fat in the form of phospholipids (Ros 2000). Triglycerides are neutral fats composed of three fatty acids of various lengths attached to a glycerol backbone. Lipids, being bulky hydrocarbon molecules, are hydrophobic and cannot mix easily with the aqueous environment of the human digestive system. Therefore, dietary fat must be digested and packaged in a way that allows the body to readily absorb the final products of fat metabolism to be used for its energy needs.

Digestion of Dietary Fat

Digestion is defined as the process whereby energy from food is transformed in such a way that the body can absorb it and use it for fuel. (Ros 2000). The first step in the digestion of any food is mastication by the teeth. The purposes of mastication are 1) to break food into smaller pieces so they are small enough for swallowing, 2) mix food with saliva 3) stimulate the taste buds, which increases salivary, gastric, pancreatic and bile secretion in preparation for further digestion (Sherwood 1997).

The digestion of dietary fat is accomplished through enzymatic hydrolysis by lipase enzymes. They are the only enzymes secreted in the digestive system that can digest lipids and are found in the mouth, stomach and duodenum. Lipase enzymes break

bonds joining fatty acid molecules to the glycerol backbone of triglycerides. The final units of triglyceride digestion are monoglycerides and free fatty acids.

Lingual lipase acts on dietary fat in the mouth. The enzyme works primarily on short and medium chain fatty acids of the triglyceride molecule. Lingual lipase plays only a small role in lipid digestion since its ideal pH is 5.4. Once the bolus reaches the stomach, the action of lingual lipase is halted in the acidic gastric environment.

Gastric lipase is the primary lipid-digesting enzyme in the stomach. It generally hydrolyzes one out of every four triglyceride molecules (Ros 2000). The stomach forms an emulsion using antral peristalsis to mix and grind its contents. This emulsion is commonly referred to as chyme. The extent of lipolysis by gastric lipase is limited. Intact triglyceride molecules remain hydrophobic and disperse to the oil phase of the chyme mixture where gastric lipase cannot interact further to promote continued lipid digestion.

Chyme is released through the pyloric sphincter into the duodenum in very small amounts allowing for complete digestion by pancreatic secretions. The presence of fatty acids, as well as amino acids and peptides from protein digestion, stimulate duodenal receptors to secrete cholecystokinin (CCK). This hormone stimulates the gallbladder to contract, releasing bile into the duodenum and stimulating pancreatic cells to release pancreatic lipase and colipase. Similarly, when the acidic contents of the stomach reach the duodenum, secretin is released by the enterocytes. This hormone stimulates the pancrease to release a bicarbonate-rich secretion that neutralizes the duodenal contents, which is essential since lipase is inactive with a pH <6.0, its optimum pH being 8.0 (Ros 2000). Also, the alkalization in the duodenum ionizes the fatty acids in the emulsion

released from the stomach, causing their polar ends to face towards the water phase on the surface of the oil droplet. This physiochemical change causes the size of fat droplets to reduce from 2-5 to $\leq$0.5 μm (Ros 2000). The smaller fat droplets helps stabilize the molecule so multiple droplets cannot coalesce again, as well as allows for increased surface area exposure for pancreatic lipase to complete lipid digestion.

Pancreatic lipase is the crucial enzyme in lipid digestion. The pancreas secretes more than enough lipase, 100-1000 times greater than what is needed for the digestion of a normal meal, indicating its importance in the processing of fat (Ros 2000). It is secreted in its active form and works to hydrolyze the ester bonds of the triglyceride in the 1 and 3 positions. The enzyme colipase works in conjunction with lipase by anchoring it to the surface of the lipid droplets and unfolding the enzyme to expose its active site for adherence to triglycerides. When secreted by the pancreas, colipase is inactive but is readily activated by the proteolytic enzyme trypsin. The final products of pancreatic lipase action on one triglyceride molecule are 2 free fatty acids and one 2-monoglyceride molecule.

Bile, released from the gallbladder, aids lipid digestion in two ways. First, bile salts help convert large lipid globules into a lipid emulsion consisting of many small fat droplets. In this way, bile also increases the surface area upon which lipase may interact with lipids. Without bile, lipid digestion would take place, but would be much slower and may occur to a lesser extent.

Secondly, and perhaps more importantly, bile prepares lipids for absorption across the unstirred water layer of the small intestine. As stated earlier, lipids are hydrophobic in nature and are unable to pass through the aqueous intestinal environment unaided.

Bile acids, amphipathic molecules with both hydrophobic and hydrophilic regions, acquire hydrophobic lipids, such as 2-monoglyceride, free fatty acids, lecithin, cholesterol and fat-soluble vitamins, which aggregate with bile acids to form small clusters called micelles. Within micelles, hydrophobic lipids are situated within the core, while hydrophilic molecules remain close to the aqueous environment. Micelles function as a shuttle for lipids, allowing them to travel easily to the brush border of the small intestine where they can be absorbed.

Bile acids work continuously down the length of the small intestine. Once they reach the ileum, they are reabsorbed via enterohepatic circulation.

Absorption of DietaryFat

Once micelles reach the luminal membranes of the epithelial cells in the distal duodenum and proximal jejunum, 2-monoglycerides and free fatty acids passively diffuse out of the micelle and into the cells lining the intestine. The pH of 5.3-6.0 in the small intestine promotes micellar dissociation and fatty acid protonation, which facilitates the diffusion across the lipid portion of the cellular membrane (Ros 2000). Micelles are then free to pick up more dietary lipids for transport. 2-monoglyceride and free fatty acids are re-synthesized to triglycerides within the interior of intestinal cells by various enzymes. Once formed, triglycerides aggregate into lipid droplets once again. The droplets are coated with a layer of lipoprotein, which create a hydrophilic coat around the triglyceride globule to form chylomicrons. Chylomicrons are pulled from the intestinal cell via exocytosis and enter the intestinal fluid within the villus of the enterocyte. Once in the villus, the chylomicron is taken up into the lymph system through the lacteals and eventually reaches the body's circulation.

Chylomicrons undergo intravascular hydrolysis at certain tissue sites, primarily adipose and muscle tissue. Lipoprotein lipase, an enzyme on the endothelial surface of small blood vessels and capillaries, releases free fatty acids and triglycerides from the interior of the chylomicron. These molecules are quickly absorbed by adipose and muscle cells. Muscle tissue uses fatty acids for fuel. Adipose tissue, on the other hand, generally resynthesizes triglycerides and stores them in adipose cells for future energy needs.

Excretion of DietaryFat

A certain degree of fat excretion is considered normal. Typical amounts can range from 3-5 g/d (Asenjo 1952, Fine et al 1992, Pederson et al 1987). Though researchers disagree regarding the origin of fecal fat, published studies support the theory that excreted fat either originates as unabsorbed dietary fat (Wollaeger et al 1947, Wollaeger et al 1953) or as secretions from the intestines (Shapiro et al 1936, Crowe et al 1956). Studies have shown the intestines to be metabolically active in the synthesis of fats and that the ileum secretes a fatty fluid. It is thought that this fluid serves as a lubricant for the intestinal tract (Crowe et al 1956). Still, studies support the belief that unabsorbed food fat is the primary source of fecal fat. A study by Wollaeger et al found that increasing dietary fat up to 350 g resulted in significant amounts of fat in stool (Wollaeger et al 1947). He concludes that the amount of fat excreted is directly related to the quantity of fat present in the diet. Conversely, Crowe et al states there should not be a sharp rise in fat excretion if there is no impairment of the absorptive capacity of the gastrointestinal tract. In summary, fecal fatty acids are likely a combination of

unabsorbed residue of fatty acids entering the gastrointestinal tract either by secretion, desquamation, bacterial synthesis and ingested food (Wollaeger et al 1953).

Diet and Weight Gain

Results from the 1999-2000 NHANES study showed that 64% of adults are either overweight or obese (CDC website 2004). Obesity has reached epidemic proportions: not only in Western cultures, but around the world. Several contributors to this global problem are thought to be the abundance and availability of high fat foods, the consumption of larger portions and the sedentary lifestyle led by individuals in many populations. The public health concern of this growing problem is important, since the risk of diseases, such as diabetes mellitus, cardiovascular disease, as well as all-cause mortality, increase in proportion to the increase in body adiposity above optimal levels (Nagao et al 2000).

Typically, weight gain occurs when total energy intake exceeds energy expenditure. Excess energy is stored as fat in adipose tissue. It seems logical that a diet rich in high fat foods would promote weight gain, since fat has 9 kcal/g versus carbohydrate and protein with only 4 kcal/g. Theoretically, choosing foods with a high fat content would, therefore, consume more kilocalories on a gram by gram basis compared to persons consuming lower fat foods. Hence, the public health message to consume a diet low in fat has become common knowledge.

As a result of the World Health Organization's Consultation on Obesity in 1999, the WHO concluded that, "the fundamental causes of the obesity epidemic are sedentary lifestyles and high-fat (30-40% of total energy intake), energy-dense diets" (WHO 1997). They now suggest lower fat intakes within the range of 20-25% for sedentary individuals.

Other than the energy density of fat, high fat diets may promote weight gain since a smaller amount of calories are used by the body to convert ingested fat into stored fat compared to the amount of calories needed to transform carbohydrate and protein to fat (Bray et al 1998). In addition, as the proportion of fat increases, carbohydrate intake decreases and results in less glucose utilization. A decrease in glucose utilization may promote greater food intake before satiety sets in resulting in greater energy consumption. Moreover, studies suggest that high fat diets raise the set point for body weight (Sherwood 1997).

Recently there has been some debate over the role dietary fat actually plays in the rising incidence of obesity. A portion of the published literature supports the WHO and concludes that high fat diets are the leading cause of obesity (Nagao et al 2000, Astrup et al 2000, Blundell et al 1999, Bray et al 1998). According to an extensive review of the literature and meta-analysis by Astrup et al, it has been clearly shown that ad libitum low fat diets prevent weight gain in subjects of normal weight and promote weight loss in overweight subjects (Astrup et al 2000). Research done by Bray and Popkin found that a 10% reduction in the proportion of energy from fat was associated with a reduction in weight of 16 g/d or 2.9 kg over 6 months, based on randomized, controlled, ad libitum low-fat, high-carbohydrate intervention studies (Bray et al 1998).

Still, literature proposes the need for further debate over the high fat vs. low fat theory (McCroy et al 2000, Gibney 1999). It has been suggested that factors other than dietary fat alone contribute to weight gain. The decline in physical activity may play a larger role than previously thought in the energy balance equation. It has been demonstrated that the rise in obesity in the United Kingdom parallels a rise in sedentary

lifestyle (Gibney 1999). It has also been noted that while obesity levels increased by 30% in the United States between 1976 and 1986, the proportion of total energy from fat fell. This seems to indicate that dietary fat is only part of the reason many people in our world continue getting heavier.

This, of course, makes logical sense. It is incorrect to believe that only one factor (dietary fat consumption) could be the sole contributor to the problem. It has been suggested that the energy density of food contributes more to weight gain than the proportion of fat in the diet. This could explain why rates of obesity continue to climb even though the percentage of fat actually consumed has decreased. Since the beginning of society's 'fat phobia' the food industry has developed methods of making the same flavorful products with little or no fat. To do this, however, sugar is frequently added to maintain the flavor. Therefore, the low fat version often has the same energy density as its high fat counterpart. The paradox to this situation is that modified foods may potentially lead to weight gain, since individuals mistake 'low fat' for low in kilocalories and interpret it as a justification to consume larger quantities of food.

A study conducted by McCroy et al examined the relationships between food's palatability, energy density and energy intake to determine whether the effects of fat on energy are independent of or determined by the high energy density of fat (McCroy et al 2000). Their metabolic study consisted of two 9-day diet phases where seven monozygotic twin pairs were fed ad libitum either high or low fat foods. Mean daily energy intake did not vary over the study phase and did not differ significantly between the low and high fat phases, despite diets being matched for energy density, palatability and fiber content. They concluded that energy density and food palatability were

significant determinants of energy intake, independent of fat content, since many energy dense foods are also more palatable (McCroy et al 2000). It is also suggested that the availability and variety of high energy foods and the fact that individuals are consuming more meals away from home contributes significantly to the rising weight of individuals in the United States.

It is important also to note briefly that genetic factors play a role in the body's response to dietary consumption. Blundell et al proposed that there are high-fat and low-fat phenotypes, predisposing individuals to select and eat particular food types. This may be plausible, since consumption of a high-fat diet does not always lead to obesity. An individual's physiological profile unquestionably plays a role in the type of diet that causes weight gain (Blundell et al 1999).

Clearly, the relationship between dietary fat and weight gain requires further investigation. Until the issue is resolved it is logical and plausible that the consumption of diets high in fat tends to cause body weight gain, since they contain more energy, gram for gram, than low fat diets.

High Fat Diets Containing Nuts: Influence on Body Weight

Nuts have been a staple of various diets around the world for centuries (Sabaté et al 2003) . It is only recently that the consumption of nuts, primarily in the United States, has declined. Perhaps this is a result of the connection between fat consumption, weight gain and the risk of certain diseases, such as cardiovascular disease, diabetes and obesity. Recently, however, nuts are gaining a reputation as a heart-healthy food by reducing the risk of cardiovascular disease (CVD) (Rajaram et al 2001, Sabaté et al 2003). Despite being a high fat food, providing approximately 73-90% of calories as fat (Sabaté 1993), a

large proportion is in the form of monounsaturated fats (MUFA). It is now universally known that diets high in MUFA can improve blood lipid profiles, thereby reducing the risk of CVD. For this reason, it can be argued that nuts have a place in healthy diets.

When increasing the consumption of nuts it can be assumed that weight gain will result without adjustment to other factors in the energy balance equation (i.e. reduction in caloric intake, physical expenditure or both). After reviewing the literature, however, this assumption may be premature. Thirteen studies on free living subjects reported either weight loss or no weight change on nut diets (Tables 3 and 4). Of seven controlled feeding studies, all stated no change in body weight, body mass index (BMI) or reported a weight loss despite controlling total caloric intake to prevent weight changes. Of particular interest are several studies that supplemented the diet with various nuts (Abbey et al 1994, Alper et al 2002, Durak et al 1999, Fraser et al 2002, Morgan et al 2000, Spiller et al 1992). Spiller et al added 100g of almonds to the diet for 9 weeks (Spiller 1992). Total fat increased from 28% to 36% of total calories ($p<0.05$) and calorie intake increased from 2113 to 2194 kcal/day. However, body weight did not change significantly during the study. Subjects in a study done by Morgan et al were randomly assigned to either control or pecan treatment group. Both were on self-selected diets, but the pecan group consumed an additional 459 kcal and 44g fat as pecans over 8 weeks (Morgan et al 2000). Although this group ingested 25,704 kcal as pecans over the course of the study (which would account for a 3.3 kg wt gain) weights did not differ from baseline (64 ± 12 vs. 64 ± 12 kg; baseline vs. week 8).

Table 1. Study Design and Characteristics of Nut-Feeding Studies

Study	Where Conducted	Study Design	Subjects
Controlled feeding studies:			
Berry et al AJCN, 1991	Israel	Randomized, controlled, crossover feeding trial	22 males
Berry et al AJCN, 1992	Israel	Randomized, controlled, crossover feeding trial	17 males
Sabaté et al N Eng J Med, 1993	California	Randomized, controlled, crossover feeding trial	18 males
Kris-Etherton et al AJCN, 1999	Pennsylvania	Randomized, controlled, crossover feeding trial	9 males, 13 females adolescents
Curb et al Arch Intern Med, 2000	Hawaii	Randomized, controlled, crossover feeding trial	15 males, 15 females
Rajaram et al J Nutr, 2001	California	Randomized, controlled, crossover feeding trial	14 males, 9 females
Sabaté et al Am J Clin Nutr, 2003	California	Randomized, controlled, crossover feeding trial	14 males, 11 females
Free-living subject studies			
Spiller et al J Am Coll Nutr, 1992	California	Dietary advice, pre-post supplemental field study	13 males, 13 females hypercholesterolemic
Abbey et al AJCN, 1994	Australia	Dietary advice, consecutive supplemental field study	16 males
Colquhoun et al Food Australia, 1996	Australia	Dietary advice, randomized crossover, field study	7 males, 7 females hypercholesterolemic
O'Byrne et al Lipids, 1997	Florida	Dietary advice, parallel arm field study	25 females, post-menopause hypercholesterolemic
Chrisholm et al EJCN, 1998	New Zealand	Dietary advice, randomized, crossover clinical study	21 males hypercholesterolemic
Spiller et al J Am Coll Nutr, 1998	California	Dietary advice, parallel arm field study	30 males and females hypercholesterolemic
Durak et al Clin Chimica Acta, 1999	Turkey	Dietary advice, pre-post supplemental field study	18 males, 12 females adolescents
Edwards et al J Am Coll Nutr, 1999	California	Dietary advice, randomized, crossover clinical study	4 males, 6 females hypercholesterolemic
Morgan et al JADA, 2000	New Mexico	Dietary advice, randomized, controlled, parallel arm study	15 females, 4 males
Zambón et al Ann Intern Med, 2000	Spain	Dietary advice, randomized, crossover clinical study	28 males, 27 females hypercholesterolemic
Fraser et al J Am Coll Nutr, 2002	California	Randomized, crossover feeding trial	81 males and females
Alper et al Int J Obes Relat Metab Disord, 2002	Indiana	Three arm, cross-over, Intervention study	Seven females, eight males, healthy
Wien et al Int J Obes Relat Metab Disord, 2003	California	A randomized, prospective 24-week trial	65 overweight and obese adults

Table 2. Dietary Characteristics of Nut-Feeding Studies

Study	Diet Period	Diet Comparisons
Controlled feeding studies:		
Berry et al	Two 12-week dietary periods	High MUFA diet (olive oil, almonds, avocado) High PUFA diet (safflower oil, soy oil and walnuts)
Berry et al	Two 12-week dietary periods	High CHO diet High MUFA diet (olive oil, almonds, avocado)
Sabaté et al	Two 4-week dietary periods	Step I diet Walnut diet (84g/2500 kcal)
Kris-Etherton et al	Five 24-day dietary periods	Peanuts/Peanut butter Peanut oil Olive oil Step II diet Average American diet
Curb et al	Three 30-day dietary periods	Typical American diet Step I diet Macadamia nut diet
Rajaram et al	Two 4-week dietary periods	Step I diet Pecan diet (72 g/2400 kcal)
Sabate et al	Three 4-week dietary periods	Step I diet Low-almond diet (34 g/2000 kcal/day) High-almond diet (68 g/2000 kcal/day)
Free-living subject studies		
Spiller et al	9 weeks	Baseline diet Almond diet (100g/d)
Abbey et al	Three 3-week dietary periods	Almond diet (84g/d) Walnut diet (68g/d) Control diet
Colquhoun et al	Two 4-week dietary periods	Pre-entry diet Macadamia nut (50-100g) Lowfat diet
O'Byrne et al	6 months	Lowfat diet Peanut based diet (35-68g/d)
Chrisholm et al	Two 4-week dietary periods	Walnut diet (78g/d) Lowfat diet
Spiller et al	Four weeks	Control diet Olive oil based (48g/d) Almond based (100g/d)
Durak et al	30 day diet period	Hazelnut diet (1g/d/kg body weight)
Edwards et al	Two 3-week dietary periods	Control diet Pistachio diet (20% daily caloric intake)
Morgan et al	8 weeks	Control diet Pecan diet (68g/d)
Zambión et al	Two 6-week dietary periods	Step I with walnuts (41-56g/d) Step I (Mediterranean) diet
Fraser et al	Two 6 month diet periods	Habitual diet Habitual diet + almonds
Alper et al	Two 8-week, one 3-week dietary periods	Free feeding + peanuts Peanut addition Peanut substitution
Wien et al	24 weeks	Almond low-calorie diet (LCD) Carbohydrate- LCD

Table 3a. Total Fat and Energy Differences of Nut-Feeding Studies

Study	Diet	Total Fat			Total Energy (kcal)			Notes
Controlled Feeding Studies								
Berry et al	High MUFA diet High PUFA diet	34% en 34			2800 2800			en = energy See attached notes
Berry et al	High CHO diet High MUFA diet	Planned 23.2% en 33.8	Analyzed 18.3% en[2] 32.5		NR			[2] Mean of duplicate analyses See attached notes
Sabaté et al	Step I diet Walnut diet	Planned 29.7% en 30.8	Observed 29.3% en 31.3		Planned 2523 2536	Observed 2583 2620		See attached notes
Kris-Etherton et al	Peanuts/Peanut butter Peanut oil Olive oil Step II diet Average American diet	36% en 34 34 25 34			NR			
Curb et al	Typical American diet Step I diet Macadamia nut diet	53% en 46 46			Planned 3211 3296 3283	Observed 3301 3426 3418		Energy intake was adjusted when necessary to maintain body weight See attached notes
Rajaram et al	Step I diet Pecan diet	Planned 29.9% en 42.1	Observed 28.3% en 39.6		Planned 2400 2400	Observed 2386 2491		Energy intake was adjusted when necessary to maintain body weight.
Sabaté et al	Step I diet Low Almond diet High Almond diet	Planned 30.7% en 35.1 39.4	Observed 29.9% en 35.0 39.0		Planned 2440 2447 2439	Observed 2422 2486 2448		Energy intake was adjusted when necessary to maintain body weight.
Free-living Subject Studies								
Spiller et al	Baseline diet Almond diet	67(6.9) g/day [2] 90(4.1)			2113(137.4) 2194(96.6)			[2] Mean(SEM) See attached notes
Abbey et al	Almond diet Walnut diet Control diet	35.9(1.2)% en [2] 36.5(1.2) 35.7(1.3)			2319(58) 2342(72) 2295(91)			[2] Mean(SEM) See attached notes
Colquhoun et al	Pre-entry diet Macadamia nut Lowfat diet	37.14(6.24)% en[1] 42.36(2.64) 21.29(2.66)			1969(621)[1] 1855(693) 1936(636)			[1] Mean(SD)
O'Byrne et al	Lowfat diet (n=13) Peanut based diet (n=12)	Before 23(6)[2] 34(5)% en	After 17(4)[3] 26(3)[3]	Δ -6 -8	Before 1690(381)[2] 1851(418)	After 1448(323) 1657(330)	Δ -242 -194	[2] Mean(SD), [3] P<0.01 See Attached Notes
Chrisholm et al	Walnut diet Lowfat diet	38(4)% en[1] 30(4)[2]			2255(586)[1] 2171(596)			[1] Mean(SD), [2] P<0.01, See Attached notes
Spiller et al	Control diet (n=12) Olive oil based (n=15) Almond based (n=18)	Baseline 33(5)g/day[3] 28(8) 34(8)	Week 4 35(4)g/day 35(4)[2] 39(5)[2]		Baseline 1852(321)[3] 2013(362) 1668(362)	Week 4 1917(257) 2183(362)[2] 1703(283)		[2] Significant within group from baseline (P<0.05) [3] Mean(SD)
Durak et al	Hazelnut diet	NR			NR			Average daily allowance of almonds was 2 oz (340 kcal), See attached notes
Edwards et al	Control diet Pistachio diet	37 % en 39			1900 1905			[1] % total energy See attached notes

Table 3b. Total Fat and Energy Differences of Nut-Feeding Studies, cont'd

Study	Diet	Total Fat		Total Energy (kcal)					Comments			
Free living subject studies, cont'd												
Morgan et al		Baseline	Wk 2	Wk 4	Wk 6	Wk 8	Baseline	Wk 2	Wk 4	Wk 6	Wk 8	Table, [2]Mean(SD), [3] P<0.05, *P<0.0.1
	Pecan diet (g/kg)	0.7(0.2)[23]	1.5(0.4)*	1.6(0.4)*	1.4(0.3)*	1.4(0.4)*	1536(348)[23]	2018(402)[3]	2065(468)[3]	1786(314)[3]	1856(452)[3]	
	Control diet (g/kg)	1.1(0.3)	0.9(0.2)*	0.9(0.3)*	0.9(0.1)*	0.9(0.2)*	1514(538)	1572(344)	1598(517)	1496(150)	1447(291)	
Zambón et al		Prescribed	Actual	Prescribed		Actual			[1] Mean(SD) [2] P = 0.116			
	Walnut diet	32.7% en	33.2(1.3)[1]	1600-2000		1824(176)[2]						
	Step I diet	30.2	31.2(1.2)			1771(152)						
Fraser et al	Habitual diet	NR		NR					Average daily allowance of almonds was 40-50 nuts (320 kcal)/day			
	Habitual diet + almonds											
Alper et al	Baseline	30.8(1.9)[1 2]		2290(150)[3 2]					[1] % energy [2] mean ± SEM [3] kcal/day 50% of dietary fat energy came from peanuts. Mean daily provision from peanuts was 505±118 kcal See attached notes			
	Free feeding + peanuts	38.8(1.2)		2460(160)								
	Peanut addition	38.8(1.6)		2510(170)								
	Peanut substitution	34.9(1.6)		2300(150)								
Wien et al	Almond-LCD°	39[1]		1012[2]					° Low-calorie diet [1] % of energy [2] kcal			
	Carbohydrate- LCD	18		1015								

Table 4a. Body Weight, BMI and Percent Body Fat of Subjects in Nut-Feeding Studies

Study	Diet	Body Weight (kg)	Body Mass index (BMI)	% Body Fat	Comments
Controlled Feeding Studies					
Berry et al	High MUFA diet High PUFA diet	NR*	Before: 23.3(2.4)[1] 21.6(2.2) After: NR	NR	[1] Mean(SD) See Attached Notes
Berry et al	High CHO diet High MUFA diet	Before: 67.3(9.2) 68.9(10.8) After: NR NR	Before: 21.4(1.6) [1] 22.4(4.1) After: NR	NR	[1] Mean(SD) See Attached Notes
Sabaté et al	Step I diet Walnut diet	Baseline: Between 60-103 kg (mean)	Baseline: Between 18.7 – 30.6 (23.8)	NR	See Attached Notes
Kris-Etherton et al	Peanuts/Peanut butter Peanut oil Olive oil Step II diet Average American diet	NR	Initial BMI of subjects was 20-27	NR	Subject's weight was maintained throughout study (± 1 kg)
Curb et al	Typical American diet Step I diet Macadamia nut diet	NR	Males: 24(2.4)[1], (19.5-27.9)[2] Females: 22(2.6), (19.1-28.3)	NR	[1] Mean BMI [2] Range See Attached Notes
Rajaram et al	Step I diet Pecan diet	NR[1]	NR	NR	[1] See Attached Notes
Sabaté et al	Baseline Step I diet Low Almond diet High Almond diet	71.0 (6.1)[1] 71.0 (.030) 71.2 (0.30) 70.7 (0.30)	<30 kg/m² NR NR NR	NR	[1] Mean (SE)
Free-living Subject Studies					
Spiller et al	Almond diet	Wk 0: 74.9 Wk 3: 74.1 Wk 6: 74.8 Wk 8: 74.1 Wk 9: 74.3	NR	NR	Only means reported No significant changes in body weight.
Abbey et al	Baseline Almond diet Walnut diet Control diet	Before: 86.1(2.8)[1] After: 85.5(2.8) 85.4(2.9) 85.8(2.9)	NR	NR	[1] Mean(SEM) No significant change in body weight throughout the study
Colquhoun et al	Pre-entry diet Macadamia nut Lowfat diet	74.82(10.58) 74.07(10.99) 74.18(10.92)	NR	NR	
O'Byrne et al	Lowfat diet (n=13) Peanut based diet (n=12)	Before: 71.0(10.5)[1] 68.6(6.2) After: 70.8(11.0) 65.3(6.5)	Before: 26.4(3.3) 26.2(3.8) After: 26.3(3.6) 24.9(3.8)	Before: 34.5(3.6) 34.6(3.6) After: 34.0(5.5) 31.5(3.5)	[1] Mean(SD) See Attached Notes
Chrisholm et al	Walnut diet Lowfat diet	87.3(15.6)[1] 87.3(15.0)	NR	NR	[1] Mean(SD)

NR*-Not Reported

Table 4b. Body Weight, BMI and % Body Fat of Subjects in Nut Feeding Studies, cont'd

Study	Diet	Body Weight (kg)					Body Mass Index (BMI)		% Body Fat			Comments
Free Living Subject Studies, cont'd												
Spiller et al	Control diet (n=12) Olive oil based (n=15) Almond based (n=18)	Baseline 64(11)[1] 69(15) 65(13)	4 weeks 63(11) 67(14) 65(13)				NR		NR			No significant change in body wt between groups at 4 weeks
Durak et al	Hazelnut diet	Before 68.7(9.2)[1]	After 69.2(10.6)[2]				NR		NR			[1] Mean(SD) [2] p>0.05
Edwards et al	Control diet Pistachio diet	NR					NR		NR			No significant changes in body weights
Morgan et al (n=19)	Control diet Pecan diet	Baseline 64(12)[1] 66(12)	Wk 2 63(10) 67(11)	Wk 4 63(9) 66(9)	Wk 6 64(11) 67(10)	Wk 8 64(12) 66(11)			Before 24(5)[2] 24(4)	After 24(5) 24(4)	NR	[1] Mean(SD) [2] BMI(SD) See Attached Notes
Zambión et al (n=55)	Baseline Walnut diet Step I diet	70.6(12.1)[1] 69.9(12.5) 70.1(12.3)	Δ -0.2 kg p = 0.07				NR		NR			[1] Mean(SD), Body weight was stable throughout the two dietary periods.
Fraser et al (n=81)	Habitual diet Habitual diet + almonds	Average weight gain was 0.40 kg (p~0..09)					NR		NR			See Attached Notes
Alper et al (n=15)	Baseline Free feeding + peanuts Peanut addition Peanut substitution	NR					NR		Pretreat NR 24.6 NR NR	Week 8 NR 25.6 NR NR		See Attached Notes
Wien et al (n=65)	Almond-LCD° (n=32) Carbohydrate-LCD (n=33)	Week 0 244.7(1.8)[1] 244.7(1.8)	Week 24 201.7(2.3) 218.1(2.2)		Week 0 38.3(0.3)[2] 38.4(0.3)	Week 24 31.6(0.3) 34.2(0.3)			NR			°Low-calorie diet [1] lbs [2] kg/m² See attached notes

Table 5. Summary of Nut Feeding Studies (Comments)

Study	Notes
Controlled Feeding Studies	
Berry et al	Average weight change of subjects from both groups in period 1 was <0.5 kg; during period 2 the change was <1.0 kg.
Berry et al	No significant change in body weight or BMI. Subjects on CHO diet during period 1 gained 1.9 kg and BMI increased by 0.6 – reason unclear. There was no evidence that subjects did not adhere to the diet during this period. Physical activity similar to MUFA group, who did not gain weight.
Sabaté et al (n=18)	Energy intake was adjusted when necessary to maintain weight. Average (SD) body weight decreased by 1.4(1.8) kg over the study. The decrease was not related to a specific diet. The mean difference between the dietary treatments in weight lost was 0.099 kg (P=0.97).
Curb et al (n=30)	Values believed to be baseline. Only 1 subject had a 1.53 kg weight loss during the study period. In a pilot study 70 free-living subjects were either given 90 g or 45 g supplements of macadamia nuts or consumed a regular diet. There was no significant change in the mean weight of any of the groups after 1 month.
Rajaram et al (n=23	Subjects lost 0.43 ± 0.18 kg ($P < 0.05$) during the pecan-enriched diet period compared with the Step I diet period.
Sabaté et al (n=25)	The high–almond diet significantly lowered body weight (-0.51 kg) compared to the low almond diet.
Free living subject studies	
Spiller et al (n=19)	It appears that the minor increase in total caloric intake from 2113 to 2194 kcal/day was not sufficient to affect weight of the subjects.
Abbey et al (n=16)	The three diet periods were well matched for total energy intake and major dietary components, as reflected in the stable body weight throughout the study.
O'Byrne et al	Subjects on peanut based diet showed continuous weight loss during the study. After following the peanut based diet for 6 months subjects lost ~ 3 kg (P< 0.01), while the LF group maintained their weight. Subjects who lost weight had difficulty consuming all the required food. Once they were eliminated from the group mean, the mean weight loss for the peanut based diet group was 2.1 kg. Weight loss was likely due to decreased energy intake. Subjects in each group chose lower fat foods than baseline. Linear regression (p=0.839), indicates that no interaction exists between the peanut based diet and weight gain. BMI decreased (P<0.01) due to weight loss and body fat was slightly lower in the peanut-based diet. Both diets were designed to maintain weight, but subjects in both groups reduced energy intake (Time Effect, P<0.01) by ~194-241 kcal.
Chrisholm et al (n=16)	Despite detailed dietary instructions and regular reinforcement throughout the experimental period, total energy from fat was higher on the walnut diet. Examination of the food records suggest that instead of replacing other high fat foods with walnuts the subjects were consuming the raw nuts in addition to their usual food. Nevertheless, the diets were essentially isocaloric and some food substitution was evident.
Spiller et al	Caloric intake was significantly higher (p<0.05) in the olive oil group compared with the almond or control group. Total fat intake increased significantly (p<0.05) in the almond and olive oil groups, but not in the control group.
Durak et al	Diet of subjects was not standardized before the study, but none of the subjects changed their dietary habits during this period. Subjects consumed 1 g/day/kg body weight of hazelnut in addition to normal diets for 30 days.
Edwards et al	Roasted, unsalted pistachios were substituted for 20% of subject's daily caloric intake.
Morgan et al	Body weights were standardized using BMI and values did not change over the study. Two women had <1 kg increase in body weight by the end of the study, but this did not affect the group mean. If the study had lasted for a longer period of time weight gain could have emerged as a result of the higher energy intakes associated with pecan supplementation. Exercise was not measured for duration and intensity and differences in energy expenditure could have been a factor to the stable BMI in the pecan treatment group.
Fraser et al	Despite 57,500 calories of almonds over 6 months, on average the weight gain was only 0.04 kg. Men gained an average of 0.65kg (p=0.01). Women on average gained 0.11 kg (p=0.79). Despite a caloric supplement that should have accounted for 6.40 kg weight gain, there was no significant change in body weight, unless thinner than average.
Alper et al	Observed body weight gain (1.0 kg) significantly lower than predicted at week 8 (p<0.01). No significant change in body weight from pretreatment to week 4, yet significant increase (0.8 kg) observed from week 4 to 8 (p<0.05). No significant change in mean percentage of body fat. During peanut addition observed body weight gain (0.6 kg) lower than predicted (p<0.05). No change in body weight during peanut substitution.
Wien et al	Almond intake was associated with greater reduction in body weight and BMI (p<0.0001). A decline in fat-free mass was observed over the study period (p<0.0001) with no difference found between study groups.

Fraser et al studied the effects of daily almond supplementation (~320 kcal/d) on body weight over a six month study period. Average body weight increased only 0.40 kg ($p>0.05$) (Fraser et al 2002).

Two controlled, metabolic feeding studies conducted at Loma Linda University reported weight loss in subjects consuming diets with either 10 or 20% isoenergetic replacement of a Step I diet with pecans or almonds (Rajaram et al 2001, Sabate et al 2003). Both studies were randomized and controlled with a crossover design. During a two-week run-in phase subjects were fed a typical American diet with 34% total energy from fat. Individuals in the pecan feeding study were randomly assigned to a Step I diet (28.3%fat) or pecan diet (39.6% fat) for four weeks. Subsequently, groups then reversed their dietary interventions and proceeded for a second 4 week period. Rajaram et al reported that despite continued adjustment of caloric intake, subjects lost 0.43 ± 0.18 kg ($P < 0.05$) compared to the Step I protocol (Rajaram et al 2001).

After the two-week adaptation period, subjects participating in the Dose Response Almond Feeding Study were randomly assigned to either a Step I diet, low almond (10% energy replacement with almonds) or high almond diet (20% energy replacement with almonds). Each diet was consumed in a crossover fashion for 4 weeks. Results demonstrated that subjects on the high-almond diet lost a significant amount of weight (0.51 kg; $P \leq 0.01$) (Sabate et al 2003).

Investigators at Loma Linda University sought to determine why subjects required continued upward adjustment of caloric intake during the pecan and almond phases of their study, as well as understand why weight was lost on diets containing such a large proportion of fat. Questions arose as to the digestion and absorption of nuts; perhaps the

total amount of dietary fat consumed is not completely absorbed and is subsequently excreted in stool. After careful examination of the literature, several possibilities are proposed.

Potential Factors Affecting Digestion and Absorption of Nuts

Mastication of Hard Foods

Studies have observed the effects of chewing hard foods on masseter muscle contraction and the quantity of masticatory strokes required to pulverize foods (Jiffry et al 1983, Horio et al 1989, Shiau et al 1999). The masseter muscle provides the power used to crush and grind foods. It has been shown that the physical and chemical characteristics of food directly influence jaw muscle performance (Shiau et al 1999). In terms of the contraction of the masseter muscle during mastication of hard foods, Shiau et al determined that muscle size and force could not override the mechanoreceptor negative feedback reflex, which prevents the muscles that close the jaw from exerting extra force while chewing hard foods. He did find, however, that the muscle compensated by increasing the length of contraction while chewing. Shiau et al concluded that a longer chewing time is needed to breakdown hard foods when bite force is unchanged.

Other studies looking at the number of masticatory strokes required to pulverize hard foods found that the number of strokes depends on the hardness of foods and that the total masticatory strokes varied among individuals (Horio et al 1989). This variation may be due to habitual chewing style. Horio et al observed that despite the hardness of foods, several subjects did not chew long enough to adequately pulverize food particles. Therefore, subjects were swallowing intact particles of foods.

Form of Food

An important study by Levine et al examined how the form of a food affects digestion and absorption (Levine et al 1980). He was interested in determining how the mechanical breakdown of food affected fat absorption. Subjects consumed a vegetarian diet containing a total of 80 g of fat; 76 g of which came from whole peanuts, peanut butter or peanut oil, with equal fiber contents. He analyzed total fat in stool samples and discovered that fat absorption was directly related to the degree of refinement of peanut fat (Levine et al 1980). Not only was the amount of excreted fat greater on the whole peanut diet, visual observation of the stools showed portions of undigested nuts which bypassed digestion. Therefore, the total amount of fat in the whole peanut diet was not available for absorption. These results provide an interesting explanation as to why energy balance may be possible on diets containing nuts.

Fiber

Nuts are not only high in fat; they are a good source of dietary fiber. Almonds and pecans provide 3.5 g (per 24 nuts) and 2 g (per 15 halves) of fiber, respectively (Dreher et al 1996). Nuts supply approximately 5-10% of the recommended daily fiber intake in one 28.4 g serving (Kris-Etherton et al 1999 suppl). Although most of the fiber is in the insoluble form, approximately 25% is soluble fiber (Kris-Etherton et al 1999 suppl). Studies have demonstrated that diets high in fiber are less digestible than low-fiber diets and result in an increased fecal fat excretion (Ganji et al 1994, Lairon 1996, Miles 1992, Rumpler et al 1998). Several mechanisms are proposed, which include alteration of lipid metabolism in the stomach and small intestine. The actual process is not fully understood. However, it is believed that fiber, especially soluble fiber, increases

the viscosity of the stomach contents resulting in delayed gastric emptying. This could delay digestion and absorption of nutrients. It is also believed that a high fiber diet stimulates the release of pancreatic lipase, which may bind to fiber, particularly cellulose and xylan, thereby resulting in diminished lipid digestion. (Lairon 1996). It is questionable, however, that fiber could sequester appreciable amounts of pancreatic lipase, enough to interfere with lipid digestion, since the pancreas secretes 100-1000 times the amount needed to digest the fat in one meal (Ros 2000).

In addition, soluble fiber can change the viscosity of the contents of the small intestine. Such a change in viscosity can interfere with lipid emulsification and increase the thickness of the unstirred water layer in the intestine, negatively affecting lipid absorption (Lairon 1996). Although the fiber in nuts is primarily insoluble, the possibility that the soluble fraction has an effect on lipid digestion deserves further attention.

The physical properties of almonds, pecans and other nuts, as well as the properties of their fiber content, may diminish fat absorption. In the intestine, soluble fiber forms a continuous sol phase, combined with insoluble fiber components. The lipid component of a meal may bind with fiber making it unavailable for digestion (Miles 1992). The rate of release of insoluble particles from the sol phase is inversely related to the particle size, as well as directly proportional to the solute gradient, physical structure and surface properties of particles (Lancet 1992).

Food Structure

To date there is little published on the exact structural make-up of nuts. Ren et al has studied the microstructure of the almond cotyledon by using bright field and electron

microscopy (SEM and TEM) in hopes of identifying its main structural components, as well as the distribution of lipids and cell wall carbohydrate (Ren, unpublished). Investigators determined that carbohydrate is a primary component of the cell wall of almonds (average thickness being 0.3-1.0 μm), in addition to being present as intra-cellular deposits. Lipids are present intracellularly within the cotyledonary tissue of the almonds, with an average particle size ranging between 0.4 – 2.5 μm. It is feasible that improper breakdown and digestion of almonds may prevent the release of lipids from almond cells, thereby decreasing the amount of fat available for absorption.

Almonds

Nutrient Composition

Almonds have a total fat content of 52% (Kris-Etherton et al 1999 suppl). A majority of almond fat is in the form of monounsaturated fatty acids (Figure 1. Kris – Etherton et al 1999 suppl)

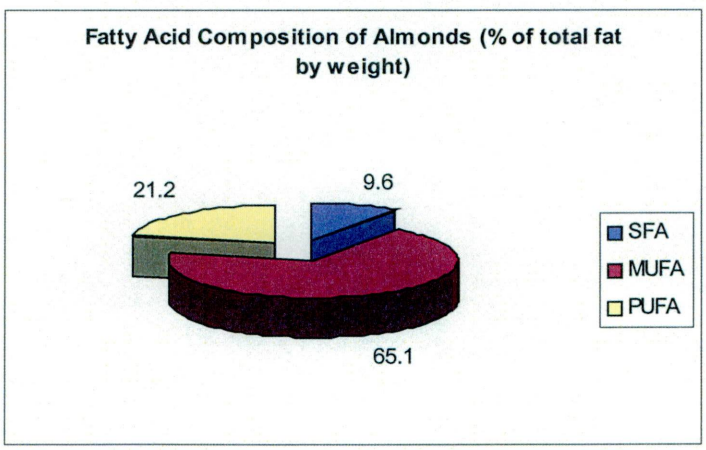

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Figure 1. Fatty Acid Composition of Almonds (% of total fat by weight)

Saturated fat is in the form of palmitic acid (6.6% of total fat by weight) and stearic acid (1.9% of total fat by weight). Oleic acid is the primary monounsaturated fat in almonds (63% of total fat by weight) and linoleic acid is the major polyunsaturated fat (20.1% of fat by total weight) (Kris-Etherton et al 1999 suppl) Almonds are also a good source of fiber, protein, vitamin e, folic acid, vitamin b6, niacin, magnesium, zinc, copper and potassium. (Dreher et al 1996).

CHAPTER THREE

MATERIALS AND METHODS

This thesis is based on a sub-study of the Dose Response Almond Feeding Study, which investigated the effects of almonds on blood lipids. I will first describe the parent study and subsequently the methodology for the stool fat sub-study.

Subjects

Nine subjects (5 females, 4 males) voluntarily participated in a second arm of the Dose Response Almond Feeding Study (Sabaté et al 2003). Age ranged from 23-66 with a mean age of 35 years. Body weight ranged from 43-84 kg (mean = 66 kg). Subject ethnicity was as follows: 4 Caucasian, 3 Hispanics, 1 Asian, 1 African-American. Subjects were recruited for this secondary study solely on a voluntary basis. Participants gave informed consent and the study protocol was approved by the Institutional Review Board of Loma Linda University.

Study Design

The study was a tightly controlled, single-blind, randomized, human feeding study (Figure 2). Following a two-week adaptation period on a Western type diet (34% energy as fat), nine healthy subjects were fed three experimental diets in a crossover fashion: control, low almond (10% energy replaced by almonds, 34 g/2000 kcal) and high almond (20% energy replaced by almonds, 68 g/2000 kcal), for four weeks each. Each diet was isoenergenic, but contained different degrees of fat (control 31%, low almond 35% and high almond 40%). The control diet followed the National Cholesterol Education Program's Step I diet. This diet included all food groups with the exception of nuts. The nutrient composition of the three diets are listed in Table 6. The intake of

monounsaturated fat, particularly oleic acid, increased with 10% and 20% caloric replacement with almonds. Linoleic acid also increased, while the amount of saturated fat remained the same on each diet period. Fiber intake also remained consistent among test diets.

Weeks:	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Run-in R	0				½				1						
	½				1				0						
	1				0				½						
	0				1				½						
	½				0				1						
	1				½				0						
Lab work	√√				√√				√√						√√

R = Randomization (allocation of subject to diet sequence). 0 = Step I diet, ½ = low almond diet, 1 = high almond diet.

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Figure 2. Study Design of the Dose-Response Study

Subjects consumed breakfast and dinner at the University Metabolic Kitchen Sunday through Friday of each week. Lunch and Saturday meals were packaged and given to subjects to consume at home or work. All meals were prepared by University staff. 10 menus were rotated throughout the study. Almonds were served whole, sliced in hot/cold foods or incorporated into recipes. 80% of almonds were served as slices, pieces or as whole almonds. 20% was consumed as almond butter or powder. Participants' body weight was measured two times per week and caloric intake adjusted accordingly to maintain stable body weights.

To ensure compliance by participants several steps were taken: 1) all meals supervised by a senior investigator, 2) a diary was maintained by each subject throughout the 14-week study. Participants recorded any variations in their diet, medications taken, as well as stool frequency and consistency. Diaries were periodically reviewed by senior investigators to determine dietary compliance and assess deviations in subjects' elimination patterns. Compliance was estimated to be nearly 100%. Please refer to the original dose response almonds feeding study for more details on the study protocol (Sabate et al June 2003).

Table 6. Planned and Analyzed Composition of the Step I, Low-almond and High-Almond Diets[1]

Nutrient	Step I diet		Low-almond diet		High-almond diet	
	Planned	Analyzed	Planned	Analyzed	Planned	Analyzed
Energy						
(kJ/d)	10195	10133	10141	10401	10090	10242
(kcal/d)	2437	2422	2424	2486	2414	2448
Protein (% of energy)	13.9	14.0	13.9	13.4	14.0	14.1
Carbohydrate (% of energy)	57.1	55.8	53.0	51.2	48.8	46.0[2]
Fat (% of energy)	31.1	29.9	35.6	35.0	40.2	39.0
SFAs (% of energy)	9.5	8.2	9.2	8.0	8.8	7.7
MUFAs (% of energy)	12.7	12.1	16.3	16.5	19.9	19.4
Oleic acid	11.6	11.6	15.2	16.0	18.9	19.3
PUFAs (% of energy)	6.3	6.2	7.5	7.5	8.7	8.7
Linoleic acid	5.1	5.7	6.4	6.9	7.8	8.1
α-Linolenic acid	0.71	0.65	0.64	0.60	0.57	0.54
Fiber (g/d)	28.1	---	29.9	---	31.9	---

[1]Planned composition was calculated with the use of FOOD PROCESSOR IV software, version 7.5 (ESHA Research, Salem, OR). Analyzed composition values were obtained from the chemical analysis of samples from the study diets. SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids.
[2]Calculated by subtracting the values for fat and protein intake from those for total energy intake.

Sample Collection

Nine volunteers participated in a 48-hour stool collection during the last week of each diet period. The collection was preceded by an exact sequence of meals. Ten menu days were used during the study in a randomized fashion. An identical menu sequence was followed during the four days proceeding and the two days of stool collection. Study participants ingested a charcoal pill as a marker. Once the charcoal was seen in the stool, collections began.

Subjects were provided with two containers and instructional booklet for sample collection and recording bowel movement time and characteristics. Each container was used to collect samples per 24-hour period. Samples were then delivered the day following collection to the laboratory. The 48-hour stool sample was weighed, recorded and homogenized and a 5 ml aliquot for each subject was frozen at -20°C until time of analysis.

Sample Analysis

Samples were analyzed by Lipomics Technologies, Inc, West Sacramento, CA for total fatty acid content, as well as individual fatty acid content, per gram of human feces using gas chromatography. Lipids were extracted from fecal samples following authentic internal standards by the method of Folch et al (Folch et al 1957) using chloroform:methanol. A 25 mg sample was used for each analysis. Individual lipid classes within each extract were separated by preparative thin-layer chromatography as described previously (Watkins et al 2001). Authentic lipid class standard compounds were spotted on the two outside lanes of the thin-layer chromatography plate to enable localization of the sample lipid classes. Each lipid fraction was scraped from the plate

and trans-esterified in 3 N methanolic-HCl in a sealed vial under a nitrogen atmosphere at 100°C for 45 min. The resulting fatty acid methyl esters were extracted from the mixture with hexane containing 0.05% butylated hydroxytoluene and prepared for gas chromatography by sealing the hexane extracts under nitrogen.

Fatty acid methyl esters were separated and quantified by capillary gas chromatography using a gas chromatograph (Hewlett-Packard model 6890, Wilmington, DE) equipped with a 30 m DB-225MS capillary column (J&W Scientific, Folsom, CA) and a flame-ionization detector as described previously (Watkins et al 2001).

Statistical Analysis

Analysis was conducted using Version 8.0 of The SAS System for Windows (copyright 1999 by the SAS Institute Inc., Cary, NC). Initial data was expressed as nmol of fatty acid per gram of stool. We then multiplied these measurements by the total mass of the stool sample to obtain the absolute amount of fatty acids (nmol) in stool. Fecal fat excretion, individual fatty acid or type of fat in response to treatment diets was analyzed by analysis of variance (ANOVA) using a mixed linear model with fixed-effect terms for diet and a random-effect term for subject. Results are expressed as least-squares means with adjustments for period effects. We again used a mixed linear model to test for dose-response relationship with a continuous covariate "DOSE" representing the percentage of calories from almonds.

CHAPTER FOUR

RESULTS

The mean values for total fat, fatty acid groups and individual fatty acids in stools are shown in Table 7. Subjects on the almond diets excreted more fat than on the control diet. The amount of total stool fat increased significantly as the percentage of energy from almonds increased (P-trend <0.001). The excretion of SFA, MUFA and PUFA increased significantly (P-trend <0.01) as almond intake increased. Excretion of individual fatty acids predominant in almonds tended to be greater on the low almond and high almond diets when compared to the Step I diet, exhibiting a dose effect as the percentage of energy from almonds increased (P-trend <0.05).

Table 8 shows mean differences between diet interventions and control for total fat, SFA, MUFA, PUFA and individual fatty acids. Total stool fat differed significantly on the high almond diet when compared to both control (P<0.001) and low almond (P<0.001) diets, but did not change considerably from control to low almond intake. The amount of total SFA, MUFA and PUFA, palmitic, oleic and linoleic fatty acids excreted in stool on the high almond diet increased significantly compared to the control and low almond diets (P<0.05), with no significance apparent between control and low almond. Greater amounts of stearic acid were excreted on the high almond diet compared to control (P<0.01), but no appreciable difference was noted when comparing control with low almond intake or low almond intake with high almond diet. Stool weight and bowel movement frequency did not differ significantly on the almond diets versus control.

Table 7. Stool Total Fat and Fatty Acids (mmol)[1]

	Control	Low Almond	High Almond	P for trend
Total Fat	36.7 ± 19.1[2]	46.0 ± 19.7	156.7 ± 19.5	.0006
SFA	17.5± 6.8	23.7± 7.1	47.9 ± 6.9	.002
MUFA	8.1 ± 10.8	12.9 ± 11.1	65.9 ± 11.1	.001
PUFA	9.9 ± 6.1	8.4 ± 6.3	41.4 ± 6.3	.005
16:0 Palmitic acid	7.1 ± 2.1	6.1 ± 2.2	15.6 ± 2.1	.01
18:0 Stearic acid	8.4 ± 5.6	16.2 ± 5.7	30.0 + 5.7	.005
18:1n9 Oleic acid	7.2 ± 10.4	12.1 ± 10.7	62.9 ± 10.6	.001
18:2n6 Linoleic acid	5.9 ± 5.2	2.2 ± 5.4	24.1 ± 5.4	.04
22:4n6 Docosatetraenoic acid	1.6 ± 2.2	4.4 ± 2.3	16.5 ± 2.2	.0005

[1] Results derived from ANOVA with diet and period effects.
[2] Least Squares Mean ± SE.
Saturated Fatty Acid (SFA), Monounsaturated Fatty Acid (MUFA), Polyunsaturated Fatty Acid (PUFA).

Table 8. Mean Differences in Total Fat and Fatty Acids between Control, Low Almond and High Almond Diets (mmol)

Variable	Low Almond – Control	High Almond – Control	High Almond – Low Almond
Total Fat	9.3 ± 0.65	119.8 ± 0.4[3]	110.4 ± -0.2[3]
SFA	6.2 ± 0.2	30.4 ± 0.1[1]	24.1 ± -0.07[1]
MUFA	4.8 ± 0.3	57.9 ± 0.2[3]	53.0 ± -0.1[2]
PUFA	-1.5 ± 0.2	31.4 ± 0.2[2]	32.9 ± -0.08[2]
16:0 Palmitic acid	-1.0 ± 0.07	8.5 ± 0.05[2]	9.5 ± -0.02[2]
18:00 Stearic acid	7.8 ± 0.20	21.7 ± 0.1[2]	13.8 ± -0.06
18:1n9 Oleic acid	4.9± 0.3	55.7 ± 0.2[3]	50.8 ± -0.1[2]
18:2n6 Linoleic acid	-3.6 ± 0.2	18.2 ± 0.1[1]	21.9 ± -0.66[1]
22:4n6 Docosatetraenoic acid	2.8 ± 0.09	14.9 + 0.06[3]	12.1 ± -0.03[2]

• Effect difference from ANOVA controlled for period effect
·Difference of least squares means ± SE
[1]P<0.05
[2]P<0.01
[3]P<0.001
Saturated Fatty Acid (SFA), Monounsaturated Fatty Acid (MUFA), Polyunsaturated Fatty Acid (PUFA).

CHAPTER FIVE

DISCUSSION

Our results indicate that the amount of total fat excreted in stool is greater on low and high almond diets compared to a nut-free control diet. This study examined the effects of two levels of almond supplementation on stool fat excretion. We discovered a dose response relationship between the amount of energy from almonds and the amount of fat excreted in stool, with total fat excretion approximately four times greater on high-almond diet than control diet. Although weight was controlled through caloric adjustment by investigators in this study, Fraser et al demonstrated that average weight gain was 0.40 kg (P~0.09) in subjects consuming a habitual diet plus 320 kcal/day from almonds over a 6 month period (Fraser et al 2002). Our findings provide preliminary explanation as to why subjects on high fat almond diets did not gain appreciable weight.

The fatty acid profile of the stool resembles the fatty acid profile of almonds. A significant amount of MUFA, primarily oleic acid, was excreted in larger amounts on the high almond diet compared to low almond and control diets. In addition, the excretion of PUFA in the form of linoleic acid increased appreciably. Refer to Table 9 for a comparison of fatty acids in almonds, study diets (control and high-almond) and stool.

Interestingly, the excretion of SFA also increased significantly despite the fact that the amount ingested by subjects on the three test diets did not differ. It is possible that long chain unsaturated fatty acids were hydrolyzed by colonic flora to form hydroxy fatty acids (Gustafsson 1982). Oleic acid can be hydroxylated to hydroxy stearic acid, which could have contributed to the total amount of saturated fat found in stool (Phillips 1984).

Table 9. Fatty Acid Comparison between Almonds, Study Diets (Control and High-almond[a]) and Stool

	Almond[1]	Dietary[2]		Difference		Stool[3]		Difference	
		Control	HA[a]	Absolute	%Change	Control	HA	Absolute	%Change
Total Fat	50.6	80.4	106	25.6	31.8	10.4	44.5	34.1	327.9
SFA	3.9	22.0	21.2	-0.8	-3.6	4.9	13.6	8.7	177.6
MUFA	32.2	32.5	52.7	20.2	62.2	2.3	18.7	16.4	713.0
Oleic acid (18:1n9)	31.9	31.2	52.4	21.2	67.9	2.0	17.9	15.9	795.0
PUFA	12.2	16.6	23.7	7.1	42.8	2.8	11.7	8.9	317.9
Linoleic -acid (18:2n6)	12.2	15.0	22.0	7.0	46.7	1.7	6.9	5.2	305.9

[1] g/100g
[2] g/day
[3] g/24 hr
SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids

In a study by Wollaeger et al, subjects consuming triolein as the only lipid source excreted a large percentage of saturated fatty acids, when one would expect to observe greater amounts of monounsaturated fat. The authors suggest that the ingested oleic acid was saturated along its journey through the intestinal tract (Wollaeger et al 1953). Secondly, the hydroxy fatty acids produced by bacteria have been shown to be potent secretagogues and may stimulate the intestines to secrete saturated fat, however studies purporting such secretions analyzed stools for total fat content only, rather than determining the individual fatty acid profile of stool (Phillips 1984). It is highly improbable, however, that saturated fats from the aforementioned sources could account for the significant amount of excreted saturated fat seen in our study.

Table 10 lists studies examining the source of fecal fat. Two out of 4 studies conclude that most of the fat found in stools originates from endogenous fat sources, either desquamation or bacterial synthesis (Shapiro et al 1936, Crowe et al 1956). This theory is supported by studies where subjects on diets void of lipids continued to excrete fat, reaching a plateau of approximately 1.5 g per day (Wollaeger et al 1953). On the other hand, two of the four studies determined that ingested dietary fat was the primary contributor to the total amount of fecal fat (Wollaeger et al 1947, Wollaeger et al 1953). Wollaeger et al reported that as the amount of ingested fat increased (from 37% energy from fat to 66% energy from fat) there was a subsequent increase in fecal fat amounts. While it is likely that the total amount of excreted fat derives from both endogenous and exogenous lipid sources, it seems logical to conclude that the significant difference in stool fat content we found in our study primarily resulted from dietary fat, since the fatty acid profile of the stool reflected the fatty acid profile of the test diets.

Table 10. Source of Fecal Fat and Dietary Influence on Fat Excretion

Author(s)	Study Design	Subjects	Length of Diets	Diets	Results	Conclusions
Wollaeger et al Gastro, 1947	NR*	5 males, 6 females	3 days	Low fat vs. High fat diet. Average intake of low fat Diet: Fat: 101.6g (37%) Calories: 2,463. High fat diet: Fat 208g (66%), Calories: 2,823.	As the amount of ingested fat increased there was a corresponding increase in fecal fat excreted (+4.6/2.2 difference, g/d). There was a +19.0/2.0 g/d difference in the percent fecal solids that was fat between the two diets.	The amount of fat in feces of healthy, normal, human subjects is influenced by the amount of fat ingested.
Shapiro et al Am J Phys, 1936	Cross-over design	2 healthy subjects with bile fistulas	5-10 days	Fatty acids labeled with DW3 and H5	When labeled fat given, only 30-35% of the labeled fat was found in the feces. The remaining 65-70% of fat was absorbed.	Most of the fecal fat came from intestinal secretions.
Crowe et al Aust Ann Med, 1956	Cross-over design	2 patients with steatorrhea	Three 6-day periods and one 3-day period	Low fat (0-196) vs. High fat (11-38g)	As fat intake increased, fat excretion increased.	Normal individuals on average diets excrete mainly endogenous fat. In patients with steatorrhea and normal patients with a high fat intake, excreted fat is likely from both endogenous and exogenous sources.
Wollaeger et al Gastro, 1953	Cross-over design	2 normal adult males (A,B)	Four diet periods lasting 5-20 days	General mixed diet (44%, 28% fat) vs. lipid free diet vs. triolein diet (28% fat)	Fat excretion during both control periods was about the same for both subjects (9, 6g). Fat excretion decreased to a level of 1.5g/d in both A and B during lipid free diet. During triolein diet fat excretion rose above those seen during the control period. Sat, Mono and Dienoic acids were found. Sat and Mono varied most with diets.	Lipid content of feces, particularly fatty acid content, is greatly influenced by the amount of lipid in diet.

*NR=Not Reported

To date, there have been no studies examining the amount of fat excreted on nut-rich diets. We determined that significantly more fat is excreted on almond diets providing 35-40% energy as fat. We considered several mechanisms to potentially explain our findings. Initially it was believed that the fiber of almonds may influence fat digestion and absorption. Almonds provide 3.5 g of fiber per 24 nuts. Studies have demonstrated that diets high in fiber are less digestible than low fiber diets and result in increased fecal fat excretion (Lairon 1996, Rumpler et al 1998, Ganji et al 1994, Miles 1992). However, there was only a 3.8g difference in fiber content between the high-almond and control diets. It is doubtful that such a minimal difference would attribute to the significant fat malabsorption seen in this study.

Several studies have examined how the form of a food affects digestion and absorption (Levine et al 1980). Levine et al analyzed stool samples for total fat in subjects ingesting 76 g of fat in the form of whole peanuts, peanut butter or peanut oil. Results indicated that fat absorption was directly related to the degree of refinement of peanut fat (Levine et al 1980). Not only was the amount of excreted fat greater on the whole peanut diet (17.8% dietary fat compared to 7% dietary fat excreted on peanut butter and 4.5% dietary fat excreted on peanut oil), visual observation of the stools showed portions of undigested peanuts. Levine suggests that malabsorption of fat occurs to an extent when consuming dietary fat in the form of whole nuts. In our study about 80% of almonds were provided in the form of whole nuts, slices or large pieces. Almond butter and meal accounted for 16.5% of almond intake during the high almond diet period vs 13.3% on the low almond period. Since a much larger proportion of almonds were served as unrefined nuts in our study, it is possible that the form of almonds affected the

amount of fat excreted on the nut diets. Studies observing mastication of hard foods, such as nuts, offer an explanation for Levine's observation of intact peanut particles in stool of subjects consuming whole nuts. Several studies have observed the effects of chewing hard foods on masseter muscle contraction and the quantity of masticatory strokes required to pulverize foods (Jiffry et al 1983, Horio et al 1989, Shiau et al 1999). It has been shown that the physical and chemical characteristics of food directly influence jaw muscle performance (Shiau et al 1999). Shiau et al determined that muscle size and force could not override the mechanoreceptor negative feedback reflex, which prevents the muscle that closes the jaw from exerting extra force while chewing hard foods. He did find, however, that the muscle compensated by increasing the length of contraction. Shiau concluded that a longer chewing time is needed to breakdown hard foods when force bit is unchanged (Shiau et al 1999).

Other studies looking at the number of masticatory strokes required to pulverize foods found that the number of strokes depends on the hardness of foods and that total masticatory strokes varied among individuals (Horio et al 1989). This variation could be due to habitual chewing style. Horio et al (Horio et al 1989) observed that despite the hardness of foods, several subjects did not chew long enough to adequately pulverize food particles. Therefore, subjects ingested foods that were not completely broken down. These mastication studies could potentially explain why subjects on high-fat nut diets did not gain weight. If nuts, being a relatively hard food, were not chewed adequately in the mouth before being swallowed, large particles of these foods would pass through the digestive tract. Nutrients in these particles, lipids being of primary interest, would be unavailable for digestion, thereby decreasing the amount of energy actually absorbed.

To date there is little published on the exact structural make-up of nuts. Ren et al (Ren et al, unpublished)) has studied the microstructure of the almond cotyledon by using bright field and electron microscopy in hopes of identifying the main structural components, as well as the distribution of lipids and cell wall carbohydrate (Ren et al, unpublished). Investigators determined that carbohydrate is a primary component of the cell wall of almonds (average thickness being 0.3-1.0 μm), in addition to being present as intra-cellular deposits. Lipids are present intracellularly within the cotyledonary tissue of the almonds, with an average particle size ranging between 0.4 – 2.5 μm. Given that incomplete mastication of almonds may occur, it is then questionable whether the intracellular lipid is released. If lipid remains confined in almond cells it would be unavailable for absorption and would not contribute to the total caloric intake of the diet ingested.

Although it was not determined whether almond particulate could be visually observed in stool samples from our study, evidence from the literature suggests that malabsorption may occur through insufficient mastication and the inherent structural makeup of the almond cell fat, thus resulting in a decrease in the absolute amount of energy provided by the diet.

The results of this study indicate that 10-20% isoenergetic replacement of control diet with almonds results in greater excretion of fat. Our study also shows that fat excreted in stools resembles the fatty acid profile of almonds, which indicates that some almond fat bypasses absorption by the gastrointestinal tract. These findings may explain why subjects consuming high-nut diets do not gain appreciable amounts of body weight.

CHAPTER SIX

SUMMARY AND CONCLUSIONS

This study examined the effects of two levels of almond supplementation on fecal fat excretion. We discovered a dose response relationship between the amount of energy from almonds and the amount of fat excreted in stool. Total stool fat differed significantly on the high almond diet when compared to both control and low almond diets, but did not change considerably from control to low almond intake. Excretion of individual fatty acids predominant in almonds tended to be greater on the low almond and high almond diets when compared to the Step I diet, exhibiting a dose effect as the percentage of energy from almonds in the diets increased. Our results indicate that malabsorption of lipids from almonds may occur, perhaps due to insufficient mastication or the inherent structural makeup of the almond cell, thus resulting in a decrease in the absolute amount of energy absorbed.

Our study provided subjects with 0, 10 and 20% isoenergetic replacement of the Step I diet with almonds in a controlled environment at the University's metabolic kitchen over three, 4-week dietary treatments. Our study was not designed to test the long-term effects of almond supplementation on body weight and fat excretion. Recent research, however, shows that supplementing the habitual diet with 320 kcal of almonds per day does not lead to significant weight gain (Fraser et al 2002). These results, in conjunction with our findings, warrant further research examining the long-term effects of almond supplementation on fecal fat excretion.

Exclusion/inclusion criteria were based on primary outcome measures of blood lipids and lipoproteins. Future studies examining fat excretion on almond-rich diets might choose to exclude individuals with conditions known to induce steatorrhea, such as chronic alcohol intake, mucosal diseases or history of gastric resection (Shils, Olson, Shike and Ross, 1999, pg 56) In addition, subjects with poor dentition should be excluded, since mastication of nuts may be compromised.

Non-compliance with the dietary protocol was minimized through incorporation of the run-in phase, the use of food diaries and the presence of a senior researcher at mealtimes. The possibility exists that subjects did not completely collect or store stools according to protocol, non-compliance with stool collections was lessened by giving two containers to subjects.

Future Research

Our study lays the groundwork for future research in the area of almond fat absorption. Several studies have examined supplementation of the diet with high-fat nuts and their effect on body weight (Abbey et al 1994, Alper et al 2002, Durak et al 1999, Fraser et al 2002, Morgan et al 2000, Spiller et al 1992). In general, body weight does not significantly change on diets with a large percentage of energy coming from nuts. Future studies should begin to look more closely at the exact mechanism contributing to the malabsorption of almond fat as seen in this study. It is hopeful that future studies will be able to conclusively identify almond fat in stool, perhaps through the use of biomarkers. Studies should determine exclusion/inclusion criteria that could potentially affect levels of fat excretion. Study designs should also develop sample collection protocol specific for stool.

The results of this study and any future studies could potentially provide answers which could alleviate the public's concern regarding high-fat nut diets and weight gain. Incorporating nuts into a healthy diet is known to reduce the risk of cardiovascular disease. Public health messages encouraging heart-healthy nut consumption without fear of gaining weight would not only allow individuals to consume a delicious, beneficial food, but to would prove to be an exciting result for scientists in the area of nut research.

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